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Abstract: INTRODUCTION Glioblastoma is a primary brain tumor with a poor prognosis despite multimodal therapy including surgery, radiotherapy and alkylating chemotherapy. Novel therapeutic options are therefore urgently needed; however, there have been various drug failures in late-stage clinical development. The proteasome represents a key target for anti-cancer therapy as successfully shown in multiple myeloma and other hematologic malignancies. AREAS COVERED This review article summarizes the preclinical and clinical development of proteasome inhibitors in the context of glioblastoma. EXPERT OPINION Early clinical trials with bortezomib ended with disappointing results, possibly because this agent does not cross the blood-brain barrier. In contrast to bortezomib and other proteasome inhibitors, marizomib is a novel drug that displays strong inhibitory properties on all enzymatic subunits of the proteasome and, most importantly, crosses the blood-brain barrier, making it a potentially very active novel agent against intrinsic brain tumors. While preclinical studies have demonstrated significant anti-glioma activity, its clinical benefit has yet to be proven. Exploiting the biological effects of proteasome inhibitors in combination with other therapeutic strategies may represent a key next step in their clinical development.

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Proteasome inhibition for the treatment of glioblastoma

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Abstract

Introduction

Glioblastoma is a primary brain tumor with a poor prognosis despite multimodal therapy including surgery, radiotherapy and alkylating chemotherapy. Novel therapeutic options are therefore urgently needed but the last years have been characterized by various drug failures in late-stage clinical development.

Areas covered

The proteasome represents a key target for anti-cancer therapy as successfully shown in the field of multiple myeloma and other hematologic malignancies, including mantle cell lymphoma where several drugs which interfere with the enzymatic activity of the proteasome have been approved and used both as single agents as well as in combinatorial regimens. This review article summarizes the preclinical and clinical development of proteasome inhibitors in the context of glioblastoma.

Expert opinion

Proteasome inhibitors have been assessed in preclinical glioma models for more than 15 years. However, early clinical trials with bortezomib, the first clinically approved proteasome inhibitor in multiple myeloma, ended with disappointing results, as this agent does not cross the blood-brain barrier, which precluded further clinical development in glioblastoma. In contrast to bortezomib and other proteasome inhibitors, marizomib is a novel drug that displays strong inhibitory properties on all enzymatic subunits of the proteasome and, most importantly, crosses the blood-brain barrier, making it a potentially very active novel agent against intrinsic brain tumors. While preclinical studies of marizomib have demonstrated significant anti-glioma activity, its clinical benefit has yet to be proven in glioblastoma. Exploiting the biological effects of proteasome inhibitors in combination with other therapeutic strategies such as immunotherapy or other targeted approaches may represent a key next step in their clinical development.

1. Background

Gliomas are primary brain tumors, which are supposed to develop from neuroglial progenitor cells. The most frequent and most malignant subtype is glioblastoma [1]. For many decades, these tumors have been diagnosed based on histopathologic features such as the presence of cells undergoing mitosis, necrotic areas and microvascular proliferation. However, in the last decade, the molecular alterations underlying the biology of gliomas have been extensively characterized. Accordingly, the current edition of the World Health Organization (WHO) classification of tumors of the central nervous system (CNS) now comprises molecular markers to describe gliomas more specifically [2]. Most importantly, the identification of mutations in the *isocitrate dehydrogenase* (IDH) 1 and 2 genes has resulted in a re-classification of diffuse gliomas. While these mutations are frequent in lower-grade gliomas, they are only present in approximately 5-10% of glioblastomas [3]. In contrast to the increased understanding of glioblastomas and other brain tumors at the genetic and molecular level, therapeutic progress has been limited and the prognosis of patients affected by glioblastoma remains poor. In contemporary clinical trials, the median survival is typically in the range of 16 to 18 months while it remains only approximately 12 months in population-based studies [4, 5].

1.1 Standard of care and recent drug development for glioblastoma

Patients with a cerebral lesion suspicious for a glioblastoma typically undergo a neurosurgical resection, which allows obtaining tissue that subsequently can be analyzed, and results in a definitive diagnosis. Maximum safe surgical resection, that is, removing as much of the tumor tissue as possible without causing new neurological deficits, has been considered the best surgical approach for glioblastoma. Beyond surgery, treatment for most patients with newly diagnosed glioblastoma is largely standardized and comprises radiotherapy with concomitant treatment with temozolomide followed by maintenance therapy with temozolomide for up to 6 cycles (TMZ/RT→TMZ) [6]. Elderly and frail patients may require adaptions in the treatment plan [5, 7]. Because of the limited efficacy of the

currently available treatment options, several approaches have been tested in patients with newly diagnosed or recurrent glioblastoma including various targeted agents [8]. The addition of tumor-treating fields (TTFields) to maintenance therapy with temozolomide prolonged progression-free (PFS) and overall survival (OS) [9, 10]. Bevacizumab prolonged progression-free but not overall survival when added to standard temozolomide-based radiochemotherapy or to lomustine in newly diagnosed or recurrent glioblastoma, respectively [11, 12, 13]. In elderly patients, the addition of bevacizumab to radiotherapy did not prolong overall survival compared to RT [14]. Approaches that were also examined in randomized phase 3 trials but considered futile include the integrin inhibitor cilengitide [15], the EGFRvIII-targeting vaccine rindopepimut in patients with newly diagnosed glioblastoma [16] and the PD-1 inhibitor nivolumab in the recurrent setting [17].

The numerous drug failures in late stage clinical development indicate that novel therapeutic approaches are urgently needed. Following their success against multiple myeloma, drugs designed to inhibit the activity of the proteasome, a central hub of many cellular processes, have also attracted interest as a novel treatment option against glioblastoma. The following summarizes important developments in the field of proteasome inhibition with a special focus on the evaluation of this treatment strategy against glioblastoma.

1.2 The ubiquitin-proteasome pathway

Degradation of proteins is a complex and strictly regulated process within a cell. The vast majority of proteins that are degraded enter the ubiquitin-proteasome pathway (UPP). Therefore, the UPP is a central regulator of various cellular processes including, but not limited to, cell survival, cell cycle progression, gene transcription, antigen presentation and DNA repair [18]. Proteins, which are no longer needed, misfolded or damaged, are subject to degradation by the UPP. To this end, a chain of exactly defined destruction steps is initiated which involves the ubiquitin activating enzyme 1 (E1), several ubiquitin-conjugating enzymes (E2) as well as the ubiquitin-protein ligases (E3). Ubiquitin is activated in an ATP-dependent

manner and subsequently shuttled by E2 and E3 enzymes to the candidate protein [19]. The successive attachment of many ubiquitin moieties results in polyubiquitylation of the target protein. The polyubiquitin chain represents a signal for directing the tagged protein to the degradation process that is performed by the 26S proteosomal complex, which resides in the nucleus and cytoplasm of all eukaryotic cells. The 26S proteasome is composed of 20S core particles, which are linked to 1 or 2 19S units. The latter mediate cleavage of the ubiquitin molecules, which are subsequently recycled whereas the target protein is degraded by the 20S core, which contains 3 catalytic activities: the caspase-like which is located in the β1 subunit, the trypsin-like (β2 subunit) as well as the chymotrypsin-like activity (β5 subunit) [20]. All enzymatic activities recognize specific motifs, which include hydrophobic residues for the chymotrypsin-like activity, basic residues for the trypsin-like activity and acidic residues for the caspase-like activity [21]. The immunoproteasome represents an alternative variant of the proteasome. Its generation and activity is induced upon various stimuli such as tumor necrosis factor (TNF)-α or interferon (IFN)-γ signaling. In the immunoproteasome, several subunits of the 20S core are changed compared to the standard 20S unit, and the 19S particle is replaced by an 11S unit. Functionally, the immunoproteasome allows the degradation of proteins to peptides which are subsequently presented at the cell surface in a MHC class I-dependent manner [22].

1.3 Proteasome inhibitors as anti-cancer agents

On account of its central role in the biology of many cancer cells, the proteasome has long been considered a promising target for anti-tumor therapy [23]. The chymotrypsin-like activity is typically targeted by hydrophobic and therefore more cell-permeable inhibitors as compared to agents that interfere with the activity of trypsin- or caspase-like sites. Because of this, most available proteaseome inhibitors mainly inhibit the chymotrypsin-like activity but have less effect on the other 2 enzymatic sites [24]. On a functional level, proteasome inhibition leads to a stabilization of the NF-κB complex in the cytoplasm. Consequently, the

expression of genes that are controlled by NF-κB is impaired. Many tumor cells such as multiple myeloma cells require intact NF-κB signaling to maintain proliferation and viability [25]. A reduction of misfolded protein clearance disturbs homeostasis of the endoplasmic reticulum, leading to cellular stress and impaired survival [26]. Inhibition of the proteasome may also increase the stability of tumor suppressor proteins such as p27 and p53, which reduces the proliferation of affected cells [27]. Taken together, drugs that interfere with the activity of the UPP may disrupt various cellular processes and lead to a stop of proliferation or the induction of cell death. In line with this assumption, initial in vitro data showed that exposure to drugs which inhibit the proteasome led to a halt in proliferation as well as the induction of apoptosis in a broad panel of cancer cells derived from hematological as well as solid tumors [28, 29]. Subsequently, preclinical in vivo testing demonstrated anti-tumor activity in leukemia and lymphoma models [30]. Proteasome inhibition may also interfere with the function of the immunoproteasomes and subsequent antigen presentation on MHC molecules. However, if this mechanism plays a relevant role in vivo and in the context of brain tumors has yet to be determined [31]. Among the preclinically available proteasome inhibitors, relatively few have reached clinical development (see below) due to a variety of reasons, including limited activity, lack of specificity, or insufficient stability.

Bortezomib

Bortezomib was identified from a screening of several boronic acid peptide small molecule analogues that were tested against a panel of tumor cell lines [32]. It acts as a reversible inhibitor of the proteasome's chymotrypsin-like activity but has limited activity against the other enzymatic activities of the proteasome. Following extensive preclinical testing, the drug was the first proteasome inhibitor to enter clinical trials. Bortezomib was particularly active against multiple myeloma and was systemically investigated in early clinical studies followed by trials with larger patient cohorts in late and early relapse followed by newly-diagnosed disease. Upon successful completion of both phase 2 and phase 3 studies, the drug was approved by the FDA and other regulatory authorities for the treatment of patients with

relapsed multiple myeloma [33, 34, 35] and later for patients with newly diagnosed disease [36]. Furthermore, the drug was subsequently approved for the treatment of patients with mantle cell lymphoma [37]. In contrast to its strong clinical activity against hematological malignancies as well as a substantial body of literature describing convincing anti-tumor activity in various preclinical models, bortezomib displayed minimal or no activity against solid tumors in larger trials [38]. Bortezomib is mainly metabolized through the liver. Side effects include nausea, diarrhea, fatigue, thrombocytopenia and peripheral neuropathy [39]. The latter may originate from a non-proteasome-dependent mechanism with non-selective binding properties of the drug, including serine proteases and other molecules [40].

Carfilzomib

Carfilzomib was approved by the FDA for the treatment of patients with relapsed and refractory multiple myeloma in 2012 [41]. Mechanistically, carfilzomib is a selective and potent irreversible inhibitor of the β5 subunit of the 20S proteasome in the epoxyketone class. Furthermore, it specifically blocks the LMP7 subunit of the immunoproteasome. In multiple myeloma, carfilzomib has been tested as a single agent but also extensively in combination with other drugs such as dexamethasone and lenalidomide showing impressive activity [42]. Carfilzomib does not cross the blood–brain barrier and is much less likely to induce peripheral polyneuropathy than bortezomib [43]. However, in contrast to other proteasome inhibitors, the drug has been linked to significant but infrequent cardiovascular adverse events, vascular toxicity, pulmonary injury, and renal toxicity. [44].

Ixazomib

In contrast to bortezomib and carfilzomib, ixazomib is orally bio-available. Ixazomib obtained FDA approval in combination with lenalidomide and dexamethasone in patients with relapsed multiple myeloma [45]. It is in the boronate peptide class and mainly acts as a reversible inhibitor of the chymotrypsin-like activity of the 20S proteasome [46]. In line with other proteasome inhibitors, exposure of multiple myeloma cells to ixazomib induces marked

caspase-dependent apoptosis [47]. Treatment-associated adverse events include gastrointestinal symptoms, dermatological side effects and neuropathy, albeit less than bortezomib, and unlike carfilzomib there is little or no significant vascular or cardiac toxicity described. [38].

Marizomib

Marizomib, initially known as NPI-0052 or salinosporamide A, is produced by the marine bacteria Salinispora tropica and Salinispora arenicola. It was discovered by researchers from the Scripps Institution of Oceanography in La Jolla, CA [48]. The drug is an irreversible inhibitor in the beta-lactone class that binds to all catalytic moieties of the proteasome (specifically β1, β2, β5) with IC50 values in the low to mid nanomolar range [49]. Administration of marizomib to patients with advanced solid tumors and hematological malignancies led to a functional inhibition of all proteasome subunits in peripheral blood mononuclear cells, with the most pronounced effect on the chymotrypsin-like activity [50]. Similar to other proteasome inhibitors, marizomib displays strong anti-cancer activity in vitro and in preclinical tumor models. Exposure of leukemia cells to marizomib led to caspase 8 and reactive oxygen species (ROS)-dependent apoptosis [51]. Following clinical testing in patients with multiple myeloma and other hematological malignancies, marizomib has also been studied in the context of glioblastoma (see below). In contrast to other proteasome inhibitors, marizomib crosses the blood-brain barrier, making it an attractive therapeutic option for tumors in the CNS. In this context, marizomib was administered to patients with CNS involvement of multiple myeloma [52]. Therapeutic activity was observed, which formed the basis for its further assessment in CNS tumors [53]. The toxicity profile of marizomib differs from other proteasome inhibitors and includes fatigue, nausea, headache, gait disturbances as well as visual and auditory hallucinations, but the drug is otherwise generally well tolerated. Adverse events associated with the CNS may be attributed to the ability of the drug to cross the blood-brain barrier and further strengthened the rationale for its evaluation in CNS disease.

2. Proteasome inhibitors in neuro-oncology

2.1 Activity of proteasome inhibitors in preclinical glioma models

Several proteasome inhibitors were never tested in patients but their preclinical assessment defined their mechanism of action and suggested efficacy against glioma cells. Among the first tested drugs in this setting was MG132, a reversible proteasome inhibitor, which induced apoptosis in several human glioma cell lines [54]. Mechanistically, MG132 promoted mitochondrial depolarization, led to an activation of JNK and p38 and interfered with the PI3K/Akt pathway. Importantly, MG132 also exerted synergistic effects with several chemotherapeutic agents in glioma cells [55].

A proteasome inhibitor known as SC68896 also displayed strong anti-glioma activity in vitro. It reduced the proliferation of glioma cells which was associated with an accumulation of p21 and p27 proteins and cell cycle arrest and induced caspase cleavage and apoptosis. SC68896 sensitized glioma cells to death stimuli such as tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and CD95 ligand, a finding that was also observed with other proteasome inhibitors in glioma stem cell cultures as well as in other tumors [56]. Administration of SC68896 prolonged the survival of glioma-bearing mice upon intraperitoneal or intra-tumoral administration [57]. Similar to other potent proteasome inhibitors pre-clinically, clinical drug development was not further pursued by the manufacturer.

Among the proteasome inhibitors that have reached clinical development and achieved approval in other malignancies, bortezomib is a prominent example and was first-in-class. Importantly, it has been extensively tested against glioma cell lines where it induced cell death and sensitized tumor cells to TRAIL [58, 59, 60]. While bortezomib significantly reduced the viability of glioma stem-like cells in vitro, no such effect was observed in neural stem/progenitor cells [61]. In contrast to these promising in vitro data, no growth inhibition

was seen in two glioma xenograft models [62]. One study suggested that exposure of glioma cells to bortezomib had no impact on the methylation status of the MGMT promoter, but reduced MGMT RNA and protein levels resulting in a sensitization towards temozolomide. Furthermore, proteasome activity in intracranial gliomas was reduced by bortezomib and the combination of bortezomib and temozolomide prolonged the survival of glioma-bearing mice compared to monotherapy with temozolomide [63]. The combination of bortezomib with the anti-angiogenic agent bevacizumab had stronger therapeutic activity in glioma-bearing nude mice compared to either treatment alone [64]. Finally, bortezomib has been used repeatedly to sensitize tumor cells to other treatment modalities including immunotherapy. Preclinically, the therapeutic activity of an oncolytic virus was enhanced by combined administration with bortezomib in a mouse glioma model. This effect may rely on increased NK cell activity but also enhanced production of mitochondrial JNK and ROS phosphorylation in tumor cells [65]. In this context, this anti-glioma activity of the clinically approved proteasome inhibitor carfilzomib has also been assessed in vitro. Preclinically, the drug reduced the viability of glioma cells in the nanomolar range and impaired both migration and invasiveness [66].

More encouragingly, exposure of glioma cells to marizomib induced striking caspase-dependent cell death. Furthermore, the drug reached orthotopically growing gliomas in mice and reduced the proteasomal activity in the tumor [67]. Subsequent preclinical assessments confirmed the blood-brain barrier-crossing properties of marizomib. Specifically, in studies involving rats and monkeys, marizomib levels in the brain were approximately 30% of those achieved in plasma [68]. The baseline chymotrypsin-like activity in the brain of monkeys was significantly reduced supporting a drug effect. Administration of marizomib to glioma-bearing mice prolonged their survival and subsequent analyses in mice using microdialysis probes implanted in the brain demonstrated changes in neurotransmitter levels upon intravenous treatment with marizomib. Brain samples collected at different time points after treatment showed that the activity of all 3 proteasome subunits was significantly reduced in the mouse

brain by marizomib with a most pronounced reduction of the chymotrypsin-like activity, again supporting the potential for a therapeutic effect [69].

2.2 Proteasome inhibitors in clinical trials for glioblastoma patients

Bortezomib, the first clinically approved proteasome inhibitor, was explored in several smaller trials in glioblastoma patients. An early phase 1 trial aimed at defining the side effects and maximum tolerated dose (MTD) of bortezomib in patients with recurrent malignant glioma. Adverse events included thrombocytopenia, neuropathy and fatigue [70]. In a single-arm study that enrolled 24 patients with newly diagnosed glioblastoma, bortezomib was added to TMZ/RT→TMZ. Median PFS was 6.2 months and median OS 19.1 months. Compared to historical controls, a benefit was assumed for patients with MGMT-methylated as well as MGMT-unmethylated tumors and no unexpected toxicity was observed [71]. In a "window of opportunity" trial, treatment with bortezomib was initiated in patients with recurrent glioblastoma before re-resection. Higher drug concentrations were found in the tumor tissue than in plasma [72]. However, due to disappointing results with all patients experiencing tumor progression within 6 months, this concept was not further pursued. A phase 2 trial exploring the safety and activity of bortezomib with the histone deacetylase (HCAC) inhibitor vorinostat in patients with recurrent glioblastoma was closed following an interim analysis demonstrating futility, with no patient being progression-free at 6 months [73]. Similarly, the combination of bortezomib with other drugs such as bevacizumab or tamoxifen in patients with recurrent glioblastoma failed to show therapeutic activity [74, 75].

In order to test the safety of marizomib in patients with recurrent glioblastoma, a phase 1 trial (MRZ-108, NCT02330562) was initiated. Bevacizumab-naïve patients with first or second relapse were treated with bevacizumab and marizomib using a 3+3 dose-escalation design. An expansion cohort at a dose level of 0.8 mg/m^2 with 24 patients was added upon definition of the MTD. Marizomib was administered at days 1, 8, and 15, bevacizumab at days 1 and 15 of a 28 days cycle. The median age of the enrolled patients was 55 years. The most

frequent treatment-related adverse events included fatigue, headache, hallucination, confusion and ataxia. One grade 5 adverse event (intracranial hemorrhage) was attributed to bevacizumab. Overall, 36 patients were included in the intent-to-treat population and 30 patients could be evaluated by RANO criteria. The overall response rate was 39% and PFS at 6 months was 39%. Of 14 patients with MGMT-unmethylated tumors, 7 achieved a PR or better, and the PFS-6 rate in the subgroup of patients with unmethylated tumors was 49% [76]. Compared to patients receiving single agent bevacizumab, these data suggest a superior activity of the combination of marizomib and bevacizumab [77]. In patients with recurrent glioblastoma treated within the MRZ-108 study, the chymotrypsin-like activity in PBMC was almost completely inhibited 1 hour after the administration of marizomib. However, chymotrypsin-like activity levels normalized prior to the next marizomib administration 7 days later [69].

In the MRZ-112 study (NCT02903069), marizomib was assessed in patients with newly diagnosed glioblastoma in combination with standard TMZ/RT→TMZ. The trial aimed at determining the recommended dose (RD) for further studies. Patients received marizomib either in combination with RT and concomitant TMZ (TMZ/RT+MRZ→TMZ+MRZ) or only together with maintenance temozolomide (TMZ/RT→TMZ+MRZ) in a dose-escalation 3+3 design. Following definition of the RD, a dose-expansion cohort for 20 patients receiving TMZ/RT+MRZ at RD → TMZ+MRZ was opened as well as a separate cohort of patients receiving TMZ/RT→TMZ+MRZ in combination with TTFields. MRZ was administered intravenously on days 1, 8, 15, 29, 36 during RT and days 1, 8, 15 during a TMZ+MRZ cycle. Overall, 66 patients were enrolled with a median age of 58 years and 50% of them receiving corticosteroids at baseline. In line with the results of the MRZ-108 study, fatigue and nausea were the most frequent adverse events, followed by hallucination, headache, confusion and ataxia. Adverse events affecting the central nervous system proved reversible and generally manageable typically being self-limiting within a few days. For the 35 patients receiving MRZ

with TMZ/RT→TMZ, the median OS was 14.8 months with 7 patients still on treatment at the time of the analysis [78]. The addition of TTFields did not result in unexpected toxicity.

Based on the clinical data obtained so far, a decision was taken to proceed with the clinical investigation of marizomib in glioblastoma patients in a pivotal randomized phase 3 trial (EORTC 1709, MIRAGE, NCT03345095). The trial aims at assessing the activity of marizomib in patients with newly diagnosed glioblastoma when added to standard temozolomide-based radiochemotherapy. The experimental treatment is compared to standard radiochemotherapy (Figure 1). A total of 750 patients will be randomized, and the primary endpoint of the trial is overall survival. Marizomib is therefore the clinically most advanced proteasome inhibitor in neuro-oncology, and the results of the EORTC 1709 trial will define if marizomib-mediated proteasome inhibition exerts a therapeutic benefit in glioblastoma patients.

2.3 Challenges and outlook

High-dimensional characterization of tumor tissue has allowed for a detailed understanding of the molecular biology of many brain tumors including glioblastoma. However, therapeutic progress has been limited and since the addition of temozolomide to the standard of care, no other drug has been established as a standard treatment. The preclinical activity of proteasome inhibitors and the availability of "next-generation" brain-penetrant drugs such as marizomib warrants their investigation in clinical neuro-oncology. However, despite their strong anti-tumor activity in vitro, the clinical activity of proteasome inhibitors has so far been mainly limited to multiple myeloma. The therapeutic activity of these drugs as single agents may be reduced over time as tumor cells may become resistant. Several mechanisms which may contribute to therapy resistance have been proposed, particularly in the context of multiple myeloma [79, 80]. These include point mutations in proteasome subunits rendering them insensitive to pharmacological inhibition as well as increased expression of components of the UPP [81, 82]. Higher expression levels of anti-apoptotic proteins and a

reduced expression of pro-apoptotic proteins have also been observed [26]. Furthermore, changes in cell proliferation, the activation of autophagy and other pro-survival signaling pathways may play a role in some tumor cells [83, 84, 85]. The ongoing assessment of proteasome inhibitors in neuro-oncology therefore needs to be accompanied by appropriate translational studies, which will help gain greater insight in the biological activity of these drugs as well as develop rational combinations and any limitations associated with their use.

3. Expert opinion

Drug development in the area of glioblastoma has been characterized by various setbacks in the recent years. Among the drugs that have failed in late-stage clinical development are several targeted agents (e.g., the integrin inhibitor cilengitide) and anti-angiogenic drugs such as bevacizumab or cediranib. Furthermore, various immunotherapeutic approaches have resulted in disappointing outcomes including the peptide vaccine rindopepimut as well as the PD-1 inhibitor nivolumab. Strategies that have shown to improve overall survival of glioblastoma patients so far include radiotherapy as well as alkylating agents such as temozolomide and lomustine [6, 86, 87]. Targeting the proteasome may represent a promising strategy as it does not work through a single point of action such as the inhibition of one pathway but critically impairs various cellular mechanisms. The biochemical properties of novel proteasome inhibitors like marizomib, which allow them to cross the blood-brain barrier, represents an important prerequisite for the successful treatment of intrinsic brain tumors. Similar to the situation with glioblastoma in adults, diffuse midline gliomas represent a major therapeutic challenge in pediatric patients. A comprehensive series of sequential quantitative high-throughput screens of more than 2700 approved and investigational drugs suggested the combination of the HDAC inhibitor panobinostat and marizomib as a promising combination which was subsequently confirmed in patient-derived xenograft models [88]. Based on this and similar observations, the clinical investigation of proteasome inhibitors with appropriate drug partners is clearly warranted.

Article highlights box

- The ubiquitin-proteasome pathway is a key regulator of many cellular processes
- Inhibition of the proteasome results in anti-proliferative and pro-apoptotic effects in glioma cells
- A clinical benefit derived from proteasome inhibitors has not yet been demonstrated in larger clinical trials of treatment for glioblastoma patients
- Marizomib is a novel, brain-penetrant pan-proteasome inhibitor which is currently being explored in clinical neuro-oncology
- Proteasome inhibition may be exploited in combination with other therapeutic strategies such as immunotherapy or molecular targeted agents to further enhance clinical benefit

Figure legend

Design of the EORTC 1709 trial (MIRAGE). GTR, gross total resection; Gy, gray; i.v., intravenous; KPS, Karnofsky performance score; p.o., per os.

Declaration of Interest

PR has received honoraria for lectures and advisory board participation from Bristol-Myers Squibb, Debiopharm, Medac, Merck, Novocure, QED and Roche and research support from MSD and Novocure.

WM has nothing to disclose.

PGR has served on advisory committees for Takeda and BMS/Celgene and has also received research funding from Takeda and BMS/Celgene.

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References

1. Ostrom QT, Cioffi G, Gittleman H, et al. CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2012-2016. Neuro Oncol. 2019 Nov 1;21(Supplement_5):v1-v100. doi: 10.1093/neuonc/noz150. PubMed PMID: 31675094; PubMed Central PMCID: PMCPMC6823730.

2. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol. 2016 Jun;131(6):803-20. doi: 10.1007/s00401-016-1545-1. PubMed PMID: 27157931.

3. Parsons DW, Jones S, Zhang X, et al. An integrated genomic analysis of human glioblastoma multiforme. Science. 2008 Sep 26;321(5897):1807-12. doi: 10.1126/science.1164382. PubMed PMID: 18772396; PubMed Central PMCID: PMCPMC2820389.

4. Gramatzki D, Dehler S, Rushing EJ, et al. Glioblastoma in the Canton of Zurich, Switzerland revisited: 2005 to 2009. Cancer. 2016 Jul 15;122(14):2206-2215. doi: 10.1002/cncr.30023. PubMed PMID: WOS:000379894000013.

5. Wen PY, Weller M, Lee EQ, et al. Glioblastoma in Adults: A Society for Neuro-Oncology (SNO) and European Society of Neuro-Oncology (EANO) Consensus Review on Current Management and Future Directions. Neuro Oncol. 2020 Apr 24. doi: 10.1093/neuonc/noaa106. PubMed PMID: 32328653.

** A timely and comprehensive overview on the current management of glioblastoma.*

6. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005 Mar 10;352(10):987-96. PubMed PMID: 15758009.

7. Weller M, Le Rhun E, Preusser M, et al. How we treat glioblastoma. ESMO Open. 2019;4(Suppl 2):e000520. doi: 10.1136/esmoopen-2019-000520. PubMed PMID: 31297242; PubMed Central PMCID: PMCPMC6586206.

8. Le Rhun E, Preusser M, Roth P, et al. Molecular targeted therapy of glioblastoma. Cancer Treat Rev. 2019 Nov;80:101896. doi: 10.1016/j.ctrv.2019.101896. PubMed PMID: 31541850.

9. Stupp R, Taillibert S, Kanner A, et al. Effect of Tumor-Treating Fields Plus Maintenance Temozolomide vs Maintenance Temozolomide Alone on Survival in Patients With Glioblastoma: A Randomized Clinical Trial. JAMA. 2017 Dec 19;318(23):2306-2316. doi: 10.1001/jama.2017.18718. PubMed PMID: 29260225; PubMed Central PMCID: PMCPMC5820703.

10. Taphoorn MJB, Dirven L, Kanner AA, et al. Influence of Treatment With Tumor-Treating Fields on Health-Related Quality of Life of Patients With Newly Diagnosed Glioblastoma A Secondary Analysis of a Randomized Clinical Trial. JAMA oncology. 2018 Apr;4(4):495-504. doi: 10.1001/jamaoncol.2017.5082. PubMed PMID: WOS:000429834400011.

11. Chinot OL, Wick W, Mason W, et al. Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. N Engl J Med. 2014 Feb 20;370(8):709-22. doi: 10.1056/NEJMoa1308345. PubMed PMID: 24552318.

12. Gilbert MR, Dignam JJ, Armstrong TS, et al. A randomized trial of bevacizumab for newly diagnosed glioblastoma. N Engl J Med. 2014 Feb 20;370(8):699-708. doi: 10.1056/NEJMoa1308573. PubMed PMID: 24552317.

13. Wick W, Gorlia T, Bendszus M, et al. Lomustine and Bevacizumab in Progressive Glioblastoma. N Engl J Med. 2017 Nov 16;377(20):1954-1963. doi: 10.1056/NEJMoa1707358. PubMed PMID: 29141164.

14. Wirsching HG, Tabatabai G, Roelcke U, et al. Bevacizumab plus nypofractionated raciotnerapy versus radiotherapy alone in elderly patients with glioblastoma: tie randomized, open-label, phase II ARTE trial. Annals of Oncology. 2018 Jun;29(6):1423-1430. doi: 10.1093/annonc/mdy120. PubMed PMID: WOS:000438508100018.

15. Stupp R, Hegi ME, Gorlia T, et al. Cilengitide combined with standard treatment for patients with newly diagnosed glioblastoma with methylated MGMT promoter (CENTRIC EORTC

26071-22072 study): a multicentre, randomised, open-label, phase 3 trial. Lancet Oncol. 2014 Sep;15(10):1100-8. doi: 10.1016/S1470-2045(14)70379-1. PubMed PMID: 25163906.

16. Weller M, Butowski N, Tran DD, et al. Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial. Lancet Oncol. 2017 Oct;18(10):1373-1385. doi: 10.1016/S1470-2045(17)30517-X. PubMed PMID: 28844499.

17. Reardon DA, Brandes AA, Omuro A, et al. Effect of nivolumab vs bevacizumab in patients with recurrent glioblastoma. The CheckMate 143 phase 3 randomized clinical trial. JAMA oncology. 2020;May 21. doi: 10.1001/jamaoncol.2020.1024. Online ahead of print.

18. Teicher BA, Tomaszewski JE. Proteasome inhibitors. Biochemical pharmacology. 2015 Jul 1;96(1):1-9. doi: 10.1016/j.bcp.2015.04.008. PubMed PMID: 25935605.

19. McGrath JP, Jentsch S, Varshavsky A. UBA 1: an essential yeast gene encoding ubiquitin-activating enzyme. EMBO J. 1991 Jan;10(1):227-36. PubMed PMID: 1989885; PubMed Central PMCID: PMCPMC452634.

20. Groll M, Heinemeyer W, Jager S, et al. The catalytic sites of 20S proteasomes and their role in subunit maturation: a mutational and crystallographic study. Proc Natl Acad Sci U S A. 1999 Sep 28;96(20):10976-83. doi: 10.1073/pnas.96.20.10976. PubMed PMID: 10500111; PubMed Central PMCID: PMCPMC34229.

21. Heinemeyer W, Fischer M, Krimmer T, et al. The active sites of the eukaryotic 20 S proteasome and their involvement in subunit precursor processing. J Biol Chem. 1997 Oct 3;272(40):25200-9. doi: 10.1074/jbc.272.40.25200. PubMed PMID: 9312134.

22. Rock KL, Goldberg AL. Degradation of cell proteins and the generation of MHC class I-presented peptides. Annual review of immunology. 1999;17:739-79. doi: 10.1146/annurev.immunol.17.1.739. PubMed PMID: 10358773.

23. Gandolfi S, Laubach JP, Hideshima T, et al. The proteasome and proteasome inhibitors in multiple myeloma. Cancer Metastasis Rev. 2017 Dec;36(4):561-584. doi: 10.1007/s10555-017-9707-8. PubMed PMID: 29196868.

24. Kisselev AF, Goldberg AL. Proteasome inhibitors: from research tools to drug candidates. Chem Biol. 2001 Aug;8(8):739-58. doi: 10.1016/s1074-5521(01)00056-4. PubMed PMID: 11514224.

25. Broemer M, Meier P. Ubiquitin-mediated regulation of apoptosis. Trends Cell Biol. 2009 Mar;19(3):130-40. doi: 10.1016/j.tcb.2009.01.004. PubMed PMID: 19217783.

26. Thibaudeau TA, Smith DM. A Practical Review of Proteasome Pharmacology. Pharmacol Rev. 2019 Apr;71(2):170-197. doi: 10.1124/pr.117.015370. PubMed PMID: 30867233; PubMed Central PMCID: PMCPMC6423620.

27. Chen X, Barton LF, Chi Y, et al. Ubiquitin-independent degradation of cell-cycle inhibitors by the REGgamma proteasome. Mol Cell. 2007 Jun 22;26(6):843-52. doi: 10.1016/j.molcel.2007.05.022. PubMed PMID: 17588519; PubMed Central PMCID: PMCPMC2031223.

28. Imajoh-Ohmi S, Kawaguchi T, Sugiyama S, et al. Lactacystin, a specific inhibitor of the proteasome, induces apoptosis in human monoblast U937 cells. Biochem Biophys Res Commun. 1995 Dec 26;217(3):1070-7. doi: 10.1006/bbrc.1995.2878. PubMed PMID: 8554559.

29. Shinohara K, Tomioka M, Nakano H, et al. Apoptosis induction resulting from proteasome inhibition. Biochem J. 1996 Jul 15;317 (Pt 2):385-8. doi: 10.1042/bj3170385. PubMed PMID: 8713062; PubMed Central PMCID: PMCPMC1217499.

30. Orlowski RZ, Eswara JR, Lafond-Walker A, et al. Tumor growth inhibition induced in a murine model of human Burkitt's lymphoma by a proteasome inhibitor. Cancer Res. 1998 Oct 1;58(19):4342-8. PubMed PMID: 9766662.

31. Eskandari SK, Seelen MAJ, Lin G, et al. The immunoproteasome: An old player with a novel and emerging role in alloimmunity. Am J Transplant. 2017 Dec;17(12):3033-3039. doi: 10.1111/ajt.14435. PubMed PMID: 28719024.

32. Adams J, Palombella VJ, Sausville EA, et al. Proteasome inhibitors: a novel class of potent and effective antitumor agents. Cancer Res. 1999 Jun 1;59(11):2615-22. PubMed PMID: 10363983.

33. Orlowski RZ, Stinchcombe TE, Mitchell BS, et al. Phase I trial of the proteasome inhibitor PS-341 in patients with refractory hematologic malignancies. J Clin Oncol. 2002 Nov 15;20(22):4420-7. doi: 10.1200/JCO.2002.01.133. PubMed PMID: 12431963.

34. Richardson PG, Barlogie B, Berenson J, et al. A phase 2 study of bortezomib in relapsed, refractory myeloma. N Engl J Med. 2003 Jun 26;348(26):2609-17. doi: 10.1056/NEJMoa030288. PubMed PMID: 12826635.

35. Richardson PG, Sonneveld P, Schuster MW, et al. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. N Engl J Med. 2005 Jun 16;352(24):2487-98. doi: 10.1056/NEJMoa043445. PubMed PMID: 15958804.

*** Randomized phase 3 trial demonstrating that bortezomib is superior to high-dose dexamethasone for the treatment of patients with relapsed multiple myeloma.*

36. San Miguel JF, Schlag R, Khuageva NK, et al. Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. N Engl J Med. 2008 Aug 28;359(9):906-17. doi: 10.1056/NEJMoa0801479. PubMed PMID: 18753647.

37. Kane RC, Dagher R, Farrell A, et al. Bortezomib for the treatment of mantle cell lymphoma. Clin Cancer Res. 2007 Sep 15;13(18 Pt 1):5291-4. doi: 10.1158/1078-0432.CCR-07-0871. PubMed PMID: 17875757.

38. Manasanch EE, Orlowski RZ. Proteasome inhibitors in cancer therapy. Nat Rev Clin Oncol. 2017 Jul;14(7):417-433. doi: 10.1038/nrclinonc.2016.206. PubMed PMID: 28117417; PubMed Central PMCID: PMCPMC5828026.

** A comprehensive review on the role of proteasome inhibitors as anti-cancer agents.*

39. Richardson PG, Briemberg H, Jagannath S, et al. Frequency, characteristics, and reversibility of peripheral neuropathy during treatment of advanced multiple myeloma with bortezomib. J Clin Oncol. 2006 Jul 1;24(19):3113-20. doi: 10.1200/JCO.2005.04.7779. PubMed PMID: 16754936.

40. Arastu-Kapur S, Anderl JL, Kraus M, et al. Nonproteasomal targets of the proteasome inhibitors bortezomib and carfilzomib: a link to clinical adverse events. Clin Cancer Res. 2011 May 1;17(9):2734-43. doi: 10.1158/1078-0432.CCR-10-1950. PubMed PMID: 21364033.

41. Siegel DS, Martin T, Wang M, et al. A phase 2 study of single-agent carfilzomib (PX-171-003-A1) in patients with relapsed and refractory multiple myeloma. Blood. 2012 Oct 4;120(14):2817-25. doi: 10.1182/blood-2012-05-425934. PubMed PMID: 22833546; PubMed Central PMCID: PMCPMC4123387.

42. Groen K, van de Donk N, Stege C, et al. Carfilzomib for relapsed and refractory multiple myeloma. Cancer Manag Res. 2019;11:2663-2675. doi: 10.2147/CMAR.S150653. PubMed PMID: 31037034; PubMed Central PMCID: PMCPMC6450182.

43. Yang J, Wang Z, Fang Y, et al. Pharmacokinetics, pharmacodynamics, metabolism, distribution, and excretion of carfilzomib in rats. Drug Metab Dispos. 2011 Oct;39(10):1873-82. doi: 10.1124/dmd.111.039164. PubMed PMID: 21752943.

44. Efentakis P, Kremastiotis G, Varela A, et al. Molecular mechanisms of carfilzomib-induced cardiotoxicity in mice and the emerging cardioprotective role of metformin. Blood. 2019 Feb 14;133(7):710-723. doi: 10.1182/blood-2018-06-858415. PubMed PMID: 30482794.

45. Moreau P, Masszi T, Grzasko N, et al. Oral Ixazomib, Lenalidomide, and Dexamethasone for Multiple Myeloma. N Engl J Med. 2016 Apr 28;374(17):1621-34. doi: 10.1056/NEJMoa1516282. PubMed PMID: 27119237.

46. Tzogani K, Florez B, Markey G, et al. European Medicines Agency review of ixazomib (Ninlaro) for the treatment of adult patients with multiple myeloma who have received at least one prior therapy. ESMO Open. 2019;4(5):e000570. doi: 10.1136/esmoopen-2019-000570. PubMed PMID: 31555488; PubMed Central PMCID: PMCPMC6735670.

47. Ito S. Proteasome Inhibitors for the Treatment of Multiple Myeloma. Cancers (Basel). 2020 Jan 22;12(2). doi: 10.3390/cancers12020265. PubMed PMID: 31979059; PubMed Central PMCID: PMCPMC7072336.

48. Feling RH, Buchanan GO, Mincer TJ, et al. Salinosporamide A: a highly cytotoxic proteasome inhibitor from a novel microbial source, a marine bacterium of the new genus salinospora. Angew Chem Int Ed Engl. 2003 Jan 20;42(3):355-7. doi: 10.1002/anie.200390115. PubMed PMID: 12548698.

49. Potts BC, Albitar MX, Anderson KC, et al. Marizomib, a proteasome inhibitor for all seasons: preclinical profile and a framework for clinical trials. Current cancer drug targets. 2011 Mar;11(3):254-84. PubMed PMID: 21247382; PubMed Central PMCID: PMCPMC3712795.

50. Levin N, Spencer A, Harrison SJ, et al. Marizomib irreversibly inhibits proteasome to overcome compensatory hyperactivation in multiple myeloma and solid tumour patients. Br J Haematol. 2016 Sep;174(5):711-20. doi: 10.1111/bjh.14113. PubMed PMID: 27161872; PubMed Central PMCID: PMCPMC5084787.

51. Miller CP, Ban K, Dujka ME, et al. NPI-0052, a novel proteasome inhibitor, induces caspase-8 and ROS-dependent apoptosis alone and in combination with HDAC inhibitors in leukemia cells. Blood. 2007 Jul 1;110(1):267-77. doi: 10.1182/blood-2006-03-013128. PubMed PMID: 17356134; PubMed Central PMCID: PMCPMC1896116.

52. Badros A, Singh Z, Dhakal B, et al. Marizomib for central nervous system-multiple myeloma. Br J Haematol. 2017 Apr;177(2):221-225. doi: 10.1111/bjh.14498. PubMed PMID: 28387460.

53. Richardson PG, Zimmerman TM, Hofmeister CC, et al. Phase 1 study of marizomib in relapsed or relapsed and refractory multiple myeloma: NPI-0052-101 Part 1. Blood. 2016 Jun 2;127(22):2693-700. doi: 10.1182/blood-2015-12-686378. PubMed PMID: 27009059; PubMed Central PMCID: PMCPMC5413296.

54. Wagenknecht B, Hermisson M, Eitel K, et al. Proteasome inhibitors induce p53/p21-independent apoptosis in human glioma cells. Cell Physiol Biochem. 1999;9(3):117-25. doi: 16308. PubMed PMID: 10494025.

55. Zanotto-Filho A, Braganhol E, Battastini AM, et al. Proteasome inhibitor MG132 induces selective apoptosis in glioblastoma cells through inhibition of PI3K/Akt and NFkappaB pathways, mitochondrial dysfunction, and activation of p38-JNK1/2 signaling. Invest New Drugs. 2012 Dec;30(6):2252-62. doi: 10.1007/s10637-012-9804-z. PubMed PMID: 22367315.

56. Kahana S, Finniss S, Cazacu S, et al. Proteasome inhibitors sensitize glioma cells and glioma stem cells to TRAIL-induced apoptosis by PKCepsilon-dependent downregulation of AKT and XIAP expressions. Cell Signal. 2011 Aug;23(8):1348-57. doi: 10.1016/j.cellsig.2011.03.017. PubMed PMID: 21440622.

57. Roth P, Kissel M, Herrmann C, et al. SC68896, a novel small molecule proteasome inhibitor, exerts antiglioma activity in vitro and in vivo. Clin Cancer Res. 2009 Nov 1;15(21):6609-18. PubMed PMID: 19825946.

58. Yin D, Zhou H, Kumagai T, et al. Proteasome inhibitor PS-341 causes cell growth arrest and apoptosis in human glioblastoma multiforme (GBM). Oncogene. 2005 Jan 13;24(3):344-54. doi: 10.1038/sj.onc.1208225. PubMed PMID: 15531918.

59. Unterkircher T, Cristofanon S, Vellanki SH, et al. Bortezomib primes glioblastoma, including glioblastoma stem cells, for TRAIL by increasing tBid stability and mitochondrial apoptosis. Clin Cancer Res. 2011 Jun 15;17(12):4019-30. doi: 10.1158/1078-0432.CCR-11-0075. PubMed PMID: 21525171.

60. Koschny R, Holland H, Sykora J, et al. Bortezomib sensitizes primary human astrocytoma cells of WHO grades I to IV for tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis. Clin Cancer Res. 2007 Jun 1;13(11):3403-12. doi: 10.1158/1078-0432.CCR-07-0251. PubMed PMID: 17545549.

61. Gong X, Schwartz PH, Linskey ME, et al. Neural stem/progenitors and glioma stem-like cells have differential sensitivity to chemotherapy. Neurology. 2011 Mar 29;76(13):1126-34. doi: 10.1212/WNL.0b013e318212a89f. PubMed PMID: 21346220; PubMed Central PMCID: PMCPMC3269770.

62. Labussiere M, Pinel S, Delfortrie S, et al. Proteasome inhibition by bortezomib does not translate into efficacy on two malignant glioma xenografts. Oncol Rep. 2008 Nov;20(5):1283-7. PubMed PMID: 18949434.

63. Rahman MA, Gras Navarro A, Brekke J, et al. Bortezomib administered prior to temozolomide depletes MGMT, chemosensitizes glioblastoma with unmethylated MGMT promoter and prolongs animal survival. Br J Cancer. 2019 Oct;121(7):545-555. doi: 10.1038/s41416-019-0551-1. PubMed PMID: 31413318; PubMed Central PMCID: PMCPMC6888814.

64. Bota DA, Alexandru D, Keir ST, et al. Proteasome inhibition with bortezomib induces cell death in GBM stem-like cells and temozolomide-resistant glioma cell lines, but stimulates GBM stem-like cells' VEGF production and angiogenesis. J Neurosurg. 2013 Dec;119(6):1415-23. doi: 10.3171/2013.7.JNS1323. PubMed PMID: 24093630; PubMed Central PMCID: PMCPMC4550014.

65. Yoo JY, Jaime-Ramirez AC, Bolyard C, et al. Bortezomib Treatment Sensitizes Oncolytic HSV-1-Treated Tumors to NK Cell Immunotherapy. Clin Cancer Res. 2016 Nov 1;22(21):5265-5276. doi: 10.1158/1078-0432.CCR-16-1003. PubMed PMID: 27390350; PubMed Central PMCID: PMCPMC5093037.

66. Areeb Z, Stylli SS, Ware TM, et al. Inhibition of glioblastoma cell proliferation, migration and invasion by the proteasome antagonist carfilzomib. Med Oncol. 2016 May;33(5):53. doi: 10.1007/s12032-016-0767-3. PubMed PMID: 27098175.

67. Manton CA, Johnson B, Singh M, et al. Induction of cell death by the novel proteasome inhibitor marizomib in glioblastoma in vitro and in vivo. Sci Rep. 2016;6:18953. doi: 10.1038/srep18953. PubMed PMID: 26804704; PubMed Central PMCID: PMCPMC4726202.

68. Di K, Lloyd GK, Abraham V, et al. Marizomib activity as a single agent in malignant gliomas: ability to cross the blood-brain barrier. Neuro Oncol. 2016 Jun;18(6):840-8. doi: 10.1093/neuonc/nov299. PubMed PMID: 26681765; PubMed Central PMCID: PMCPMC4864261.

** An important dataset on the preclinical anti-glioma activity of marizomib.*

69. Bota DA, Di K, Keator DB, et al. Human functional brain imaging data support preclinical and clinical evidence that marizomib crosses the blood-brain barrier (BBB) to inhibit proteasome activity in the brain. Cancer Res. 2019:79(13 Suppl):Abstract nr 4733.

70. Phuphanich S, Supko JG, Carson KA, et al. Phase 1 clinical trial of bortezomib in adults with recurrent malignant glioma. J Neurooncol. 2010 Oct;100(1):95-103. doi: 10.1007/s11060-010-0143-7. PubMed PMID: 20213332; PubMed Central PMCID: PMCPMC3811025.

71. Kong XT, Nguyen NT, Choi YJ, et al. Phase 2 Study of Bortezomib Combined With Temozolomide and Regional Radiation Therapy for Upfront Treatment of Patients With Newly Diagnosed Glioblastoma Multiforme: Safety and Efficacy Assessment. Int J Radiat Oncol Biol Phys. 2018 Apr 1;100(5):1195-1203. doi: 10.1016/j.ijrobp.2018.01.001. PubMed PMID: 29722661.

72. Raizer JJ, Chandler JP, Ferrarese R, et al. A phase II trial evaluating the effects and intra-tumoral penetration of bortezomib in patients with recurrent malignant gliomas. J Neurooncol. 2016 Aug;129(1):139-46. doi: 10.1007/s11060-016-2156-3. PubMed PMID: 27300524.

73. Friday BB, Anderson SK, Buckner J, et al. Phase II trial of vorinostat in combination with bortezomib in recurrent glioblastoma: a north central cancer treatment group study. Neuro Oncol. 2012 Feb;14(2):215-21. doi: 10.1093/neuonc/nor198. PubMed PMID: 22090453; PubMed Central PMCID: PMCPMC3266383.

74. Odia Y, Kreisl TN, Aregawi D, et al. A phase II trial of tamoxifen and bortezomib in patients with recurrent malignant gliomas. J Neurooncol. 2015 Oct;125(1):191-5. doi: 10.1007/s11060-015-1894-y. PubMed PMID: 26285768.

75. McCracken DJ, Celano EC, Voloschin AD, et al. Phase I trial of dose-escalating metronomic temozolomide plus bevacizumab and bortezomib for patients with recurrent glioblastoma. J Neurooncol. 2016 Oct;130(1):193-201. doi: 10.1007/s11060-016-2234-6. PubMed PMID: 27502784.

76. Bota D, Desjardins A, Mason W, et al. Full enrollment results from the phase 1/2, multicenter, open-label study of marizomib (MRZ) +/- bevacizumab (BEV) in recurrent WHO grade IV malignant glioma (glioblastoma, rGBM). Neuro Oncol. 2017;19(suppl_6):vi16.

77. Taal W, Oosterkamp HM, Walenkamp AM, et al. Single-agent bevacizumab or lomustine versus a combination of bevacizumab plus lomustine in patients with recurrent glioblastoma

(BELOB trial): a randomised controlled phase 2 trial. Lancet Oncol. 2014 Aug;15(9):943-53. doi: 10.1016/S1470-2045(14)70314-6. PubMed PMID: 25035291.

78. Mason WP, Kesari S, Stupp R, et al. Full enrollment results from an extended phase I, multicenter, open label study of marizomib (MRZ) with temozolomide (TMZ) and radiotherapy (RT) in newly diagnosed glioblastoma (GBM). J Clin Oncol. 2019:37, 2019 (suppl; abstr 2021).

79. Wallington-Beddoe CT, Sobieraj-Teague M, Kuss BJ, et al. Resistance to proteasome inhibitors and other targeted therapies in myeloma. Br J Haematol. 2018 Jul;182(1):11-28. doi: 10.1111/bjh.15210. PubMed PMID: 29676460.

80. Barrio S, Stuhmer T, Da-Via M, et al. Spectrum and functional validation of PSMB5 mutations in multiple myeloma. Leukemia. 2019 Feb;33(2):447-456. doi: 10.1038/s41375-018-0216-8. PubMed PMID: 30026573.

*** This manuscript describes the induction of resistance to proteasome inhibitors through missense mutations.*

81. Oerlemans R, Franke NE, Assaraf YG, et al. Molecular basis of bortezomib resistance: proteasome subunit beta5 (PSMB5) gene mutation and overexpression of PSMB5 protein. Blood. 2008 Sep 15;112(6):2489-99. doi: 10.1182/blood-2007-08-104950. PubMed PMID: 18565852.

82. Fuchs D, Berges C, Opelz G, et al. Increased expression and altered subunit composition of proteasomes induced by continuous proteasome inhibition establish apoptosis resistance and hyperproliferation of Burkitt lymphoma cells. Journal of cellular biochemistry. 2008 Jan 1;103(1):270-83. doi: 10.1002/jcb.21405. PubMed PMID: 17516511.

83. Selimovic D, Porzig BB, El-Khattouti A, et al. Bortezomib/proteasome inhibitor triggers both apoptosis and autophagy-dependent pathways in melanoma cells. Cell Signal. 2013 Jan;25(1):308-18. doi: 10.1016/j.cellsig.2012.10.004. PubMed PMID: 23079083.

84. Markovina S, Callander NS, O'Connor SL, et al. Bortezomib-resistant nuclear factor-kappaB activity in multiple myeloma cells. Molecular cancer research : MCR. 2008 Aug;6(8):1356-64. doi: 10.1158/1541-7786.MCR-08-0108. PubMed PMID: 18708367; PubMed Central PMCID: PMCPMC2587345.

85. Kale AJ, Moore BS. Molecular mechanisms of acquired proteasome inhibitor resistance. J Med Chem. 2012 Dec 13;55(23):10317-27. doi: 10.1021/jm300434z. PubMed PMID: 22978849; PubMed Central PMCID: PMCPMC3521846.

86. Keime-Guibert F, Chinot O, Taillandier L, et al. Radiotherapy for glioblastoma in the elderly. N Engl J Med. 2007 Apr 12;356(15):1527-35. doi: 10.1056/NEJMoa065901. PubMed PMID: 17429084.

87. Herrlinger U, Tzaridis T, Mack F, et al. Lomustine-temozolomide combination therapy versus standard temozolomide therapy in patients with newly diagnosed glioblastoma with methylated MGMT promoter (CeTeG/NOA-09): a randomised, open-label, phase 3 trial. Lancet. 2019 Feb 16;393(10172):678-688. doi: 10.1016/S0140-6736(18)31791-4. PubMed PMID: 30782343.

88. Lin GL, Wilson KM, Ceribelli M, et al. Therapeutic strategies for diffuse midline glioma from high-throughput combination drug screening. Science translational medicine. 2019 Nov 20;11(519). doi: 10.1126/scitranslmed.aaw0064. PubMed PMID: 31748226.

*** Comprehensive drug screening revealing marizomib as a promising therapeutic agent for patients with diffuse midline glioma.*